Name ____

Investigation 6: Cellular Respiration

Background

Living systems require free energy and matter to maintain order, to grow, and to reproduce. Energy deficiencies are not only detrimental to individual organisms, but they cause disruptions at the population and ecosystem levels as well. Organisms employ various strategies that have been conserved through evolution to capture, use, and store free energy. Autotrophic organisms capture free energy from the environment through photosynthesis and chemosynthesis, whereas heterotrophic organisms harvest free energy from carbon compounds produced by other organisms. The process of cellular respiration harvests the energy in carbon compounds to produce ATP that powers most of the vital cellular processes. In eukaryotes, respiration occurs in the mitochondria within cells.

If sufficient oxygen is available, glucose may be oxidized completely in a series of enzyme-mediated steps, as summarized by the following reaction:

$$C_6H_{12}O_6 + 6O_{2(g)} \rightarrow 6CO_{2(g)} + 6H_2O + \text{energy}$$

More specifically:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686 \text{ kilocalories of energy/mole of glucose oxidized}$$

The chemical oxidation of glucose has important implications to the measurement of respiration. From the equation, if glucose is the energy source, then for every molecule of oxygen consumed, one molecule of carbon dioxide is produced.

In the Procedure section, you will learn how to calculate the rate of cellular respiration by using a respirometer. These measure relative volume (changes in pressure) as oxygen is consumed by germinating plant seeds. As oxygen gas is consumed during respiration, it is normally replaced by CO_2 gas at a ratio of one molecule of CO_2 for each molecule of O_2. Thus, you would expect no change in gas volume to result from this experiment. However, in the following procedure the CO_2 produced is removed by potassium hydroxide (KOH). KOH reacts with CO_2 to form the solid potassium carbonate (K_2CO_3) through the following reaction:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$$

Since the CO_2 is being removed, the change in the volume of gas in the respirometer will be directly related to the amount of oxygen consumed. Thus, as O_2 is consumed, the overall number of gas molecules in the respirometer decreases. This change, which then causes a change in volume due to the relatively constant pressure, can be used to determine the rate of cellular respiration. However, because respirometers are sensitive to changes in gas volume, they are also sensitive to changes in temperature and air pressure; thus, you need to use a control respirometer that will measure the environmental atmospheric pressure changes –without any living cells present.

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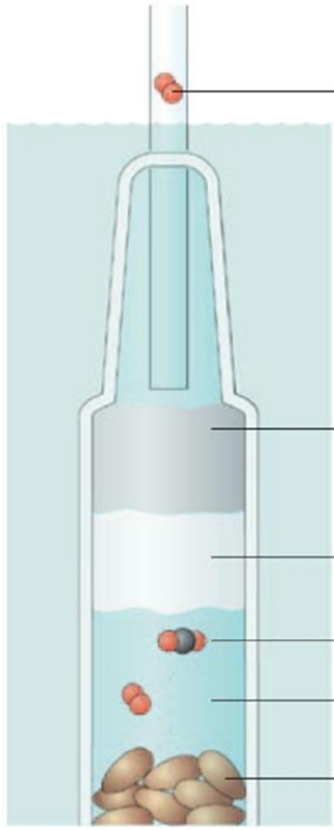

Diagram of a Microrespirometer

A number of physical laws relating to gases are important to the understanding of how the apparatus that you will use in this exercise works. The laws are summarized in the general gas law that states: **PV = nRT**

•P is the pressure of the gas,
•V is the volume of the gas,
•n is the number of moles of the gas
•R is the gas constant (its value is fixed)
•T is the temperature of the gas (in K)

This law implies the following important concepts about gases:
1. If temperature and pressure are kept constant, then the volume of the gas is directly proportional to the number of molecules of gas.
2. If the temperature and volume remain constant, then the pressure of the gas changes in direct proportion to the number of molecules of gas present.
3. If the number of gas molecules and the temperature remain constant, then the pressure is inversely proportional to the volume.
4. If the temperature changes and the number of gas molecules are kept constant, then either pressure or volume (or both) will change in direct proportion to the temperature.
5. It is also important to remember that gases and fluids flow from regions of high pressure to regions of low pressure.

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Pre-Lab Questions

Keep in mind the we will not be using the exact procedure outlined on the website below, but it will help you better understand the concept of respiration and how a respirometer works to measure the rate of respiration, as well as give you potential ideas for what variables could be studied in this lab and how to interpret your data.

- *Go to http://www.phschool.com/science/biology_place/labbench/*
- *Click on Lab 5: Cell Respiration*
- *Navigate through the concepts and explanations of the experiment to familiarize yourself with the general lab protocol by clicking "Next Concept" or "Closer Look." Where applicable, you can also click "Animate."*
- *Use the information you read in the background of the first two pages as well as the online lab bench activity to answer questions 1-4.*
- *When you get to "Analysis of Results," record your answer to the sample problem (#5) in the space provided.*
- *Finally, click "Self Quiz" to complete the lab quiz and record your answers on the appropriate pages to receive credit for them.*

1. Study the equation for cellular respiration and list three ways the rate of cellular respiration can be measured.
   -
   -
   -

2. Since we are surrounded by air AND more than one gas is involved in respiration, how can we be sure we are only measuring changes in gas volume related to the consumption of oxygen?

3. Why is it necessary to correct the readings from the seeds (such as peas) with the readings from the beads?

4. Come up with at least 3 variables that you could investigate in this lab (in regards to how they affect the oxygen consumption of seeds during respiration).
   -
   -
   -

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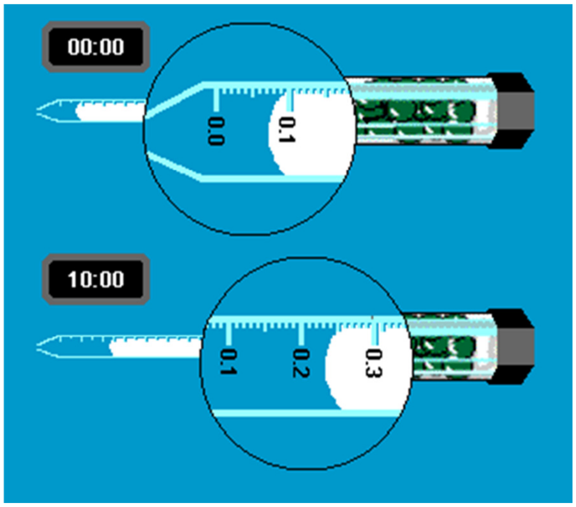
5. What would be the rate of oxygen consumption if the respirometer readings were as shown here?

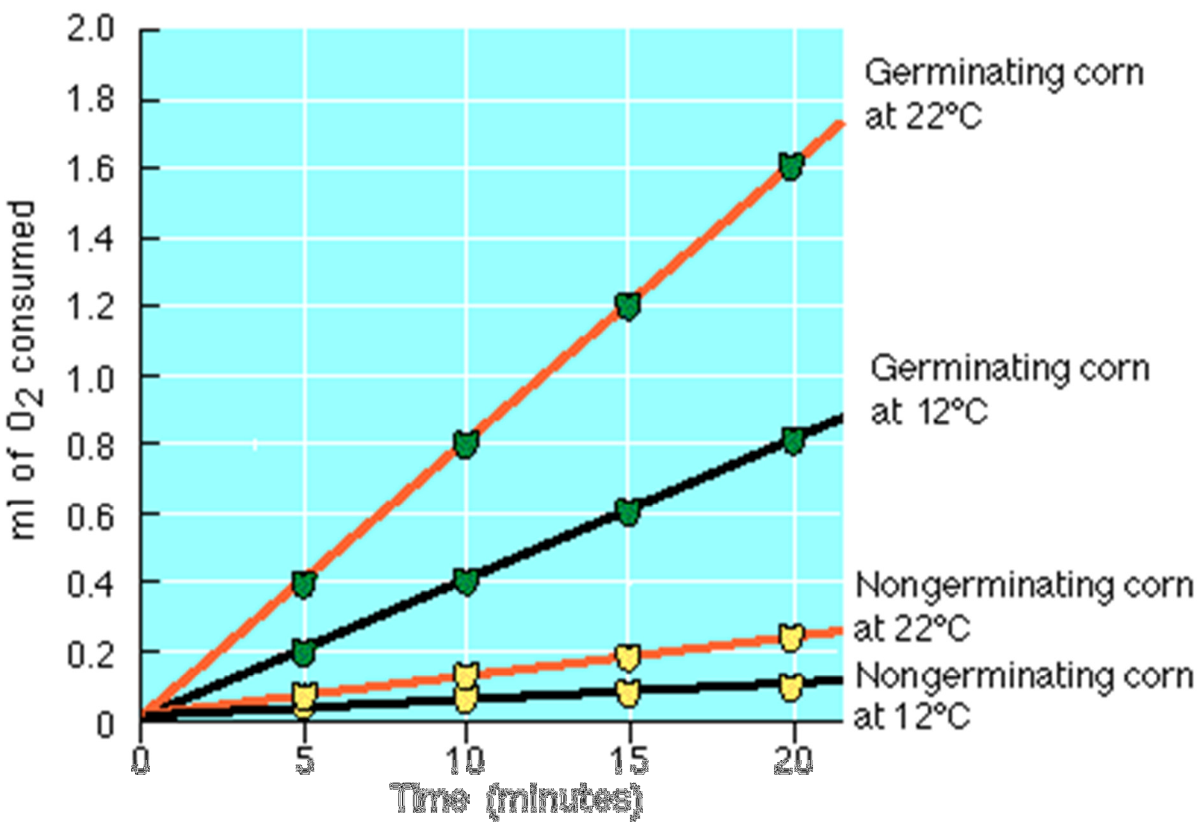


Show Your Work Here:

Your answer:

Lab Quiz

Use the graph below to answer Lab Quiz questions 1-3.



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1. Which of the following is a true statement based on the data?

- a. The amount of oxygen consumed by germinating corn at 22°C is approximately twice the amount of oxygen consumed by germinating corn at 12°C.
- b. The rate of oxygen consumption is the same in both germinating and nongerminating corn during the initial time period from 0 to 5 minutes.
- c. The rate of oxygen consumption in the germinating corn at 12°C at 10 minutes is 0.4 ml O_2/minute.
- d. The rate of oxygen consumption is higher for nongerminating corn at 12°C than at 22°C.
- e. If the experiment were run for 30 minutes, the rate of oxygen consumption would decrease.

2. What is the rate of oxygen consumption in germinating corn at 12°C?

- a. 0.08 ml/min
- b. 0.04 ml/min
- c. 0.8 ml/min
- d. 0.8 ml/min
- e. 1.00 ml/min

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3. Which of the following conclusions is supported by the data?

a. The rate of respiration is higher in nongerminating seeds than in germinating seeds.

b. Nongerminating peas are not alive, and show no difference in rate of respiration at different temperatures.

c. The rate of respiration in the germinating seeds would have been higher if the experiment were conducted in sunlight.

d. The rate of respiration increases as the temperature increases in both germinating and nongerminating seeds.

e. The amount of oxygen consumed could be increased if pea seeds were substituted for corn seeds.

4. What is the role of KOH in this experiment?

a. It serves as an electron donor to promote cellular respiration.

b. As KOH breaks down, the oxygen needed for cellular respiration is released.

c. It serves as a temporary energy source for the respiring organism.

d. It binds with carbon dioxide to form a solid, preventing CO2 production from affecting gas volume.

e. Its attraction for water will cause water to enter the respirometer.

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Hypothesis

Write a hypothesis comparing the respiration rate of your germinating vs nongerminating seeds at different temperatures:

Procedure

Preparing Identical Volumes of Seeds/Beads

1. Find the volume of 25 germinating seeds by filling a 100mL graduated cylinder with 50mL and measuring the displaced water.
2. Fill the graduated cylinder with 50mL water, 25 nongerminating seeds, and add enough glass beads to attain an equal volume to the germinating seeds.
3. Using the same procedure as in the previous two steps, find out how many glass beads are required to attain the same volume as the 25 germinating seeds.

Assembling the Respirometers

4. To assemble 3 respirometers, obtain 3 vials, each with an attached stopper and pipette.
5. Place a small wad of absorbent cotton in the bottom of each vial and, using a dropper, saturate the cotton with 15% KOH (potassium hydroxide). It is important that the same amount of KOH be used for each respirometer.
6. Place a small wad of dry, nonabsorbent cotton on top of the saturated cotton.
7. Place your identical volumes of germinating seeds, dry seeds & beads, and glass beads in their vials, keeping track of which set up is in which vial.
8. Insert the stopper with the calibrated pipette. Seal the set-up with silicone or petroleum jelly. Place a weighted collar on each end of the vial. Several washers around the pipette make good weights.

Setting Up the Water Bath Environments

9. Prepare a room-temperature bath (approx. 25^{o}C) and a cold-water bath (approx. 10^{o}C).
10. Make a sling of masking tape attached to each side of the water baths. This will hold the ends of the pipettes out of the water during an equilibration period of 7 minutes. Place your vials in the appropriate water bath.
11. After 7 min, put all three of your set-ups entirely into the water. A little water should enter the pipettes and then stop. If the water continues to enter the pipette, check for leaks in the respirometer.
12. Check the temperature in both baths and record.

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Collecting Your Data

13. Allow the respirometers to equilibrate for 3 more minutes and then record the initial position of the water in each pipette to the nearest 0.01mL (time 0) in Table 1.
14. Record the water level in the three pipettes every 5 minutes for 20 minutes.
15. Record the data from the group with an identical set up to yours but at a different temperature in Table 2.

Analyzing Your Data

16. Graph your results from the corrected difference column for the nongerminating and germinating seeds at both temperatures. (There will be 4 lines on one graph)
17. Determine the respiration rates for each seed at each temperature. (4 rates)
18. Complete the conclusions section based on your data.

Results

Table 1 Oxygen Consumption Temperature ______ºC Seed Type: _______

Time (min)	Beads Alone		Nongerminating Seeds (and Beads)			Germinating Seeds		
	Reading at time X	Diff*	Reading at time X	Diff*	Corrected Difference **	Reading at time X	Diff*	Corrected Difference **
0								
5								
10								
15								
20								

Table 2 Oxygen Consumption Temperature ______ºC Seed Type: _______

Time (min)	Beads Alone		Nongerminating Seeds (and Beads)			Germinating Seeds		
	Reading at time X	Diff*	Reading at time X	Diff*	Corrected Difference **	Reading at time X	Diff*	Corrected Difference **
0								
5								
10								
15								
20								

** difference = (initial reading at time 0) – (reading at time X)*
***corrected difference = (seed difference) – (bead difference)*

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Analysis

- Independent variable (x-axis): ___________________________________
- Dependent variable (y-axis): ___________________________________
- Include a graph of your results from Tables 1 and 2 using the corrected difference column for the nongerminating and germinating seeds at both temperatures. Reminder: There will be 4 lines on one graph
- From the slope of the four lines on the graph, determine the respiration rate of nongerminating vs germinating seeds at both temperatures and record in Table 3. Be sure to show your work.
  Remember, rate = slope = $\Delta Y / \Delta X$

Table 3

Condition	Show Slope Calculations Here	Respiration Rate (ml O_2 consumed/min)
Nongerminating Room Temp. Water Bath		
Nongerminating Cold Water Bath		
Germinating Room Temp. Water Bath		
Germinating Cold Water Bath		

Conclusion

- Explain the effect of germination (vs nongermination) on seed respiration.

- Explain the effect of temperature on seed respiration.